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[COMMUNICATION]

Tectal Visual Afferents from Fish Dorsolateral Tegmental Cells

Akiyoshi Niida and Takashi Ohono

Department of Biology, Faculty of Science, Okayama University, Okayama 700, Japan

ABSTRACT—The cells of the nucleus dorsolateralis tegmenti (NDT) in the crucian carp were physiologically identified and marked with Lucifer dye. The Lucifer dye filled axons projected into the tectum, where their main axons extended into the deep tectal layer. All the identified NDT cells responded to both optic nerve and rhombencephalic electrical stimulation. Out of 40 such NDT cells, 24 cells were visual and/or tactile. The remaining cells were unresponsive. However, some of the unresponsive cells were visually driven in conjunction with rhombencephalic electrical stimulation.

INTRODUCTION

The nucleus dorsolateralis tegmenti (NDT) in fish is located ventrolaterally to the torus semicircularis, which is considered to be a recipient of visual, auditory and lateral line inputs [1–3]. The dorsolateral tegmental area, including the NDT or deep tegmentum has been found to be reciprocally connected with the optic tectum by degeneration and HRP-labelling studies [4–9]. In a previous study [10] by means of intra-axonal dye marking and intracellular recordings we obtained the following results: (1) wide distribution of axonal branching of the NDT cells in the deep layer of the ipsilateral tectum; (2) further projection of the axon described in (1) to the contralateral tectum via the tectal commissure; (3) responses of the NDT cells to three set of electrical stimulation, optic nerve, rhombencephalon and tectal commissure. These results strongly indicate that there exist, in the tectum, afferents with various kinds of response modalities from the tegmentum. The goal of this study is thus to show the presence of visual or other sensory related responses of the NDT cells, which were identified by physiological criteria combined with cell morphology.

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MATERIALS AND METHODS

Experiments were performed on 50 crusian carp (*Carassius carassius*), 15–20 cm in overall length. The surgical procedure, methods for electrical and sensory stimulation, the recording apparatus and histological procedures have been described in detail elsewhere [10, 11]. The fish were initially anesthetized with MS-222 and immobilized with an intraperitonial injection of Flexedil. The gills were kept out of water, and perfused with aerated water through a tube inserted into the oral cavity. Beveled glass micropipettes, filled with 4% Lucifer Yellow CH (Sigma) in distilled water [12], were used for potential recording and markings. A hyperpolarizing DC current of 2 nA for 2–5 min gave good marking of the cells. The brain was removed 3–5 hr after the injection of dye and fixed for 13–15 hr with formalion acidified to pH 4.0 with acetate buffer.

The criteria for physiological identification of the NDT have been established in a previous study [10] by a combination of intra-axonal recording and Lucifer dye marking. The criteria were: 1) antidromic response of the axon, running through the stratum album centrale (SAC), to electrical stimulation (300 Hz) of the tectal commissure and

2) orthodromic response of the same axon described in (1) to electrical stimulation of both the rhombencephalon and the optic nerve. In addition to such criteria, based on a previous experiment [10], the following latency values were used for the identification of the NDT cell: 0.4–0.8 ms for the tectal commissure, 5–10 ms for the optic nerve and 1.2–1.6 ms for the rhombencephalon.

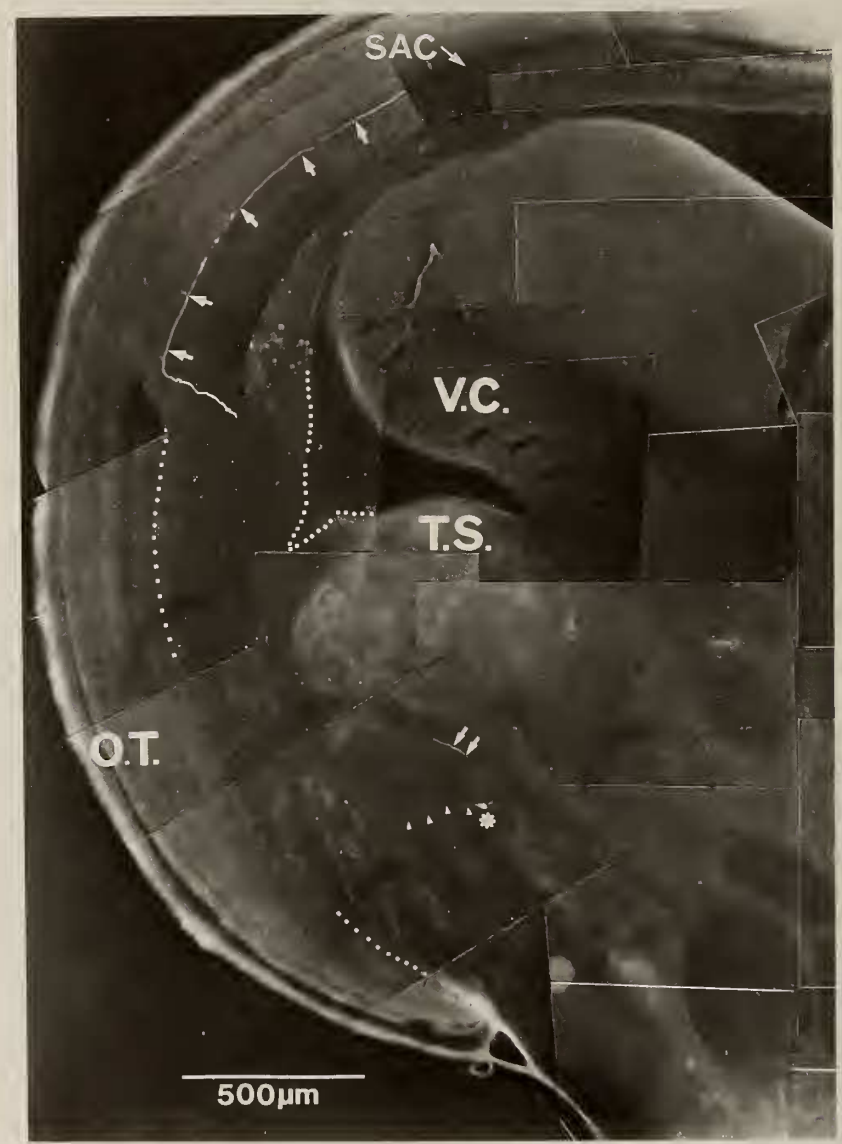

FIG. 1. Fluorescence photomicrograph of a NDT cell filled with Lucifer dye. This montage photograph was prepared by two serial coronal sections. The NDT cell is located ventrolaterally to the torus semicircularis. Dendrites (arrow heads) extend toward the tectobulbar pathway and the axon (thin arrows) arising from the soma (asterisk) ascends toward the ipsilateral tectum. The axon (thick arrows) running through SAC, the deep tectal layer could be further traced to the contralateral tectum by observing serial sections. The overall morphology of this cell is shown in Figure 2Bb. Lines of squares lateral and medial to the midline show a lower boundary of SAC and a part of the wall of the optic ventricle, respectively. The tectobulbar pathway courses downward along the line of squares lateral to the midline. Abbreviation: O.T., optic tectum; SAC, stratum album centrale; T.S., torus semicircularis; V.C., valvula cerebelli.

RESULTS AND DISCUSSION

Using above-mentioned criteria, we observed the responses of 40 NDT cells. Twenty-one of these cells, in which Lucifer dye had been injected, were well-stained (as seen from Fig. 1) and permitted tracing of the axon to the contralateral tectum. Seven of the 40 cells identified as NDT were visuo-tactile, 16 cells were visual, 1 cell was tactile and 16 cells were unresponsive.

Visuo-tactile cells

Figure 2Aa and 2Ab show one example of recordings from the bimodal NDT cells. These responses were obtained from the cell illustrated in Figure 2Ba: A spot of light (0.5 subtense angle) induced a transient response and subsequent stimuli gave a reduced number of spikes (Fig. 2Aa), indicating remarkable habituation. Simultaneously, this cell also responded to the tactile stimuli delivered by touching the facial part (stippled area in the inserted drawing) with a writing brush (Fig. 2Ab). Another example from the visuo-tactile cell following morphological identification is shown in Figure 2Ac, where normally occurring spontaneous discharges notably increased by touching the facial part. This cell responded to moving objects as well (not shown here). Bimodal units obtained by extracellular recordings have been reported by Page and Sutterlin [1] in the dorsolateral tegmentum of goldfish that are closely related to the present matrial. Unlike our results, they were all acoustico-visual units, This discrepancy is possibly due to differences in their topographycal positions where visuo-acoustic and visuo-tactile cells are located: the recording sites shown by Page and Sutterlin [1] lie more anterior to those of our cells.

Visual cells

Besides visual NDT cells coupled with tactile input, we encountered visual NDT cells with rhombencephalic inputs, which were not ascertained in response modality. They were mostly on-transient or sensitive to moving objects. As seen from Figure 2Ad, the exemplified NDT cell was directionally selective: the leading edge of a black rectangular stripe (subtense angle: 8°), moving (40°/sec) in the temporal to nasal direction through the receptive field, produced spike discharges, whereas motion in the reverse direction (nasal to temporal direction) gave a much weak response. In this example, the slightly deviated

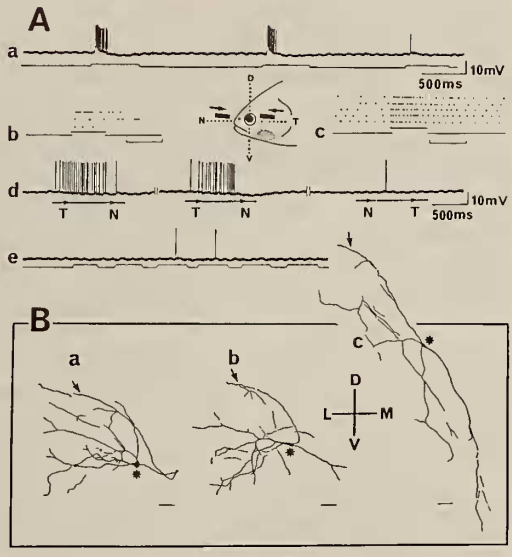

FIG. 2. A: responses of NDT cells to visual and/or tactile stimuli. (Aa) responses of the bimodal cell to a 0.5° spot of light, and (Ab) responses of the same cell to touching of the facial part (inserted drawing). Dot pattern representation of spike discharges, together with (Ac). These responses (Aa and Ab) were recorded from the cell shown in Figure 2Ba. In both responses (Aa and Ab), remarkable habituation occurs, whereas another bimodal cell (Ac) shows much less habituation with the responses induced by tactile stimuli. Their tactile receptive fields were on the facial part (stippled area in the inserted drawing). Upward deflection in each trace shows light-on for Aa and Ae, and touch for Ab and Ac. In Ab and Ac, time scale represents 1 sec. (Ad) responses of NDT cell to moving edge. When a leading edge of a black rectangular stripe moved in the temporal to nasal direction (as seen from the inserted drawing), a response was vigorously induced. A stationary spot of light to this cell was almost ineffective (Ae). These responses were recorded from the cell shown in Figure 2Bb. Calibration in (Ad) also serves for (Ae). B: composite drawings of NDT cells marked with Lucifer dye. Explanation of each cells's morphology is given in the text. Each arrow indicates axon and asterisks position of soma. Calibration bars: 50 μm. Abbreviation: D, dorsal; L, lateral; M, medial; N, nasal; T, temporal; V, ventral.

orientation of the edge from naso-temporal axis, as seen in the inserted drawing, was the most effective in the initiation of spike discharges. A moving spot of light gave no response (not shown), and a stationary spot of light was also not effective in this cell (Fig. 2Ae). The response characteristics mentioned above were obtained from the cell in Figure 2Bb, where the axon (thin arrow) filled with Lucifer dye projected into the ipsilateral optic tectum, and the dendritc field of this cell expanded in a fan-like manner toward the tectobulbar pathway (see also Fig. 1). Unlike the cell illustrated in Figure 2Ba and 2Bb, in Figure 2Bc the ventral dendrite of the NDT cell, which was sensitive to moving objects, extended toward the F.L.L. (fasciculus longitudinalis lateralis). All the identified visual cells, except for the bimodal cells, were unresponsive to acoustic and/or tactile stimuli. However, they responded to rhombencephalic electrical stimulation, indicating that these cells receive rhombencephalic inputs, such as lateral line, vestibular, and possibly proprioceptive informations.

Unresponsive cells

Among NDT cells there were some unresponsive cells in a slightly greater frequency (about 40%). They did not respond to visual, acoustic or tactile stimuli, although these cells were responsive to both optic nerve and rhombencephalic electrical stimulation. Some of these cells, however, like some of tectal efferent cells [11], which normally failed to response to acoustic, tactile, or visual stimuli, responded to light simuli under specific conditions: when continuing the rhombencephalic electrical stimulation, simultaneously applied visual stimuli induced visual responses. One of the possible interpretations for this, is that summation of visual input and heterosynaptic sensory inputs might activate responsiveness of the NDT cell. As yet neural mechanisms responsible for the unresponsiveness of these cells remain to be studied in detail. Morphological features of the unresponsive cells were substantially similar to those of visual or bimodal cells: the wide distribution of the axonal branching in the tectum, the dendritic profile extending toward the tectobulbar pathway, and the cell locations similar to those of responsive cell of the NDT.

The present study raised a crucial problem that the responses, in the deep tectum, derived from the NDT cells may be erroneously identified as intrinsic tectal unitary reponses, unless we use well-defined criteria for determining whether the responses recorded in the tectum originate from the intrinsic tectal cells or from the tegmental cells. The same appears to be the case in tectal afferents from the pretectal area and the nucleus isthmi. This situation will be overcome by a combination of more sophisticated electrical stimulation and recording techniques by which cell identification can be made.

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